...cent light microscope

ILOs (intended learning outcomes): Student should be able to use microscope for examination of bacterial stained smears.

- **Study the different parts of the microscope.**
  - The support system of the microscope (body, base and stage).
  - The illumination system (mirror, condenser and iris diaphragm).
  - The magnifying system (eye piece lens, objective lenses).
  - The adjusting system (coarse and fine adjustment knobs).
- **Magnification:**
  - The magnification achieved by a microscope is a product of the magnifying power of the eye piece and the objective lenses (low power, high power and oil immersion lenses).
  - Calculate the magnification of the microscope when using:
    - Low power lens: ....4.... x .....10.....= .....40.....times.
    - High power lens: ...10.... x .....40....= .....400...times.
    - Oil-immersion lens: ..100... x ...10.....= .....1000....times.
- **Resolution:**
  - The resolving power of the microscope is its capacity to distinguish two neighboring points as separate entities.
    - It depends on:
      1. Wave length of light
      2. Numerical aperture of objective length
    - What is the smallest size that ordinary microscope can visualize?
      200nm
    - How to examine a stained film by oil-immersion lens?
      1. Put the slide on microscope
      2. use oil over slide
      3. use the course then fine
      4. look at eye piece

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Report(2): Preparing a smear for simple staining?

ILOs: 1. To prepare a smear for staining.
2. To perform simple stain.

- How to prepare a smear for staining?
  - Sterilized bacteriologic loope by heat
  - wait for the loope to be cold quiedly
  - take a drop of water and Put it on slide
  - Sterlize the loop again
  - take a drop of a specimen by loop and in the slide and spread
  - dry the slide and fix it by flame

- List steps of simple staining by methylene blue?
  - Put methylen blue after preparation
  - wait for minute then wash it by water, Dry slide then Put a drop of oil then examine by emmersion lens

- Examine the stained smear under oil-immersion lens (OIL) of the microscope.
  - setup the microscope against a good source for light place one drop of emmersion oil on the slide Put and examinert by immersion lens
- Description of the stained bacteria.
  - Morphology: bacilli
  - Arrangement: clusters?
  - Drawing of the bacteria:

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Report (3): Gram staining

ILOs: 1. To perform Gram stain procedure.
2. To examine and identify bacteria of different morphology and Gram reaction.

- List the steps of Gram staining:
  - Cover the smear with Crystal violet or methyl violet for (30-60)sec.
  - Pour it off and with water
  - add Iodine solution and leave it to act for 1min then Pour it off and wash with water
  - DeColorize by adding 95% alcohol or Colour a kohol. Pour it off and reply till No violet Colour Comes of the smear
  - Wash rapidly with water
  - Counter stain with Safranin or dilute basic fuschin for 1min
  - Wash with water then Place the slide to air dry
- How do Gm +ve and Gm –ve bacteria behave during the following staining steps?

	Gm +ve	Gm –ve
Staining with methyl violet	✓	
Washing with alcohol	resist decolorization	decolorized
Staining with the counter stain (diluted carbol fuchsin)	violet	red

- Examine the Gm stained smear under OIL of the microscope.
  Setup the microscope facing a good source light
  Place small drop of emersion it on slide
  -Put the slide on the stage of microscope and use oil immersion lens and focus on object using fine adjustment
- Description of the Gm stained bacteria:
  - Morphology: Bacilli
  - Arrangement: ~~Clusters~~ No specific arrangement
  - Gm reaction: -ve
  - Drawing of the bacteria:

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Report (4): Sterilization and disinfection

ILOs: To examine some tools of physical method of sterilization (simple autoclave, hot air oven and seitz filter)

- **Sterilization is:** Killing of all signs of life including spores
- **Methods:**
  1. Physical → heat, radiation
  2. Chemical → disinfectant, antiseptic
  3. gases → ethylene oxide, gas plasma
  4. ~~filtration~~ filtration

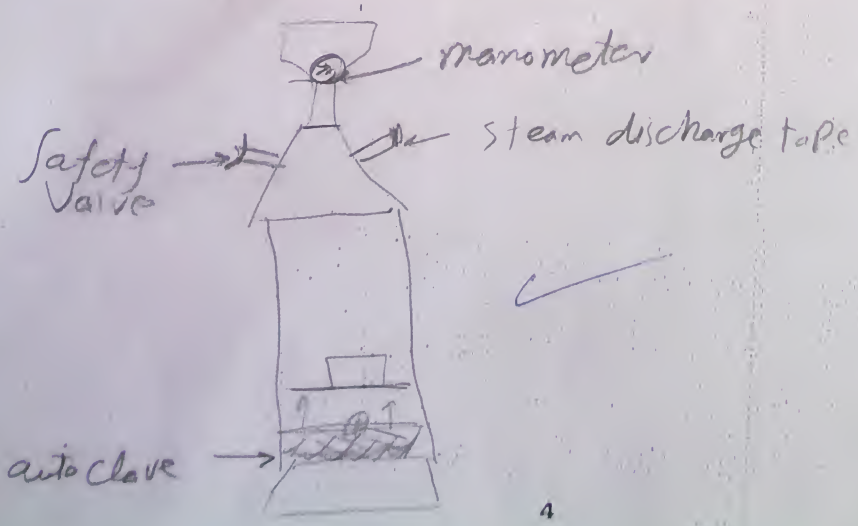
- **Outline** physical methods of sterilization.

heat
- Dry heat
  - hot air oven (Dry heat sterilizer) → anything without water
  - direct flame → loop
  - flaming → mouth of test tubes
  - incineration (burning) → disposable of medical waste
  - infrared
- Moist heat
  - < 100 Pasterization of milk
  - = 100 boiling, steam
  - > 100 steam autoclave under pressure

Radiation
- Ionizing
- ultraviolet

filtration

- **Draw a labeled diagram of simple autoclave.**



- What is the principle of plasma-gas sterilizer?
H_2O_2 alone or with Peracetic acid excited by radio freq. energy
→ free radicals toxic to bacteria

- Filtration is the suitable sterilization method for: Sterilization
biological fluids (serum, blood, Plasma)

Explain why? destroyed by heat

- Mention types of filters:
- muliPore filter (Pore size = 0.15 μm)
- Syringe filter (small volumes)
- Vaccum filter
Seitz filter
air filter (HEPA)
- Ionizing radiation method is mainly used for
Plastic devices (Gloves, Catheters, Sutures)

- Disinfection is killing of most Pathogenic organisms
not including endospores

- Mention examples of chemical disinfectants and their uses:
Protein denaturation
alcohol (ethyl alcohol) 70% – Intermediate
Phenols (Old)

inactivation of enzymes
Chlorine → gas → water
→ liquid hospital, home

aldehyde
(High level Sterilizers)
- Formaldehyde
- Glutaraldehyde

Detergent → Anionic → Soap
→ Cationic → QAC

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Report (5): Demonstration of different culture media.

ILOs: To examine and identify different culture media.

1. Simple media

- Complete the following table (1)

Medium	Main component	Use
Peptone water	Water - Peptone - Nacl	-production of other media - test for indole
N. broth	Peptone water + meat extract	-Coagulase test - blood Culture
N. agar	Peptone water + Agar	-Isolation of organism -other media

2. Enriched media: (contain highly nutritive substances as blood, serum, egg)

- Complete the following table (2)

Medium	How to prepare & sterilize	Use
Blood agar	-Steam autoclave under pressure at 121°c for 20 hrs (nutrient agar) - Blood at 55°c (Blood St. by filtration)	- Isolation of bacteria (fastidious) - as Indicator media (Type of hemolysis)
Chocolate agar	as above but blood added at 100°c (hematin)	Growth factor for Pneumococci, Neisseria (fastidious)
Loffler's serum	1.75 Serum + 1.25 glucose	Isolation of diphtheria

3. Selective media: (contain selective agent(s) that inhibits all but not the selected bacteria).

- Complete the following table (3)

Medium	Selective agent(s)	Grown bacteria
Lowenstein Jensen	Malachite green	T.B
TCBS	Potassium tellurite	diphtheria
MSA	modified chocolate agar	gonorrhoea

4. Differential (Indicator media)

- Complete the following table (4)

Medium	Test sugar	Indicator	Different colours of growing colonies
MacConkey	Lactose	neutral red	- yellow - Pink
TCBS	sucrose	bromothymol blue	- yellow - green
MSA	mannitol	phenol red	- yellow - Pink

Media for anaerobic bactetia:

- Robertson's Cooked meat
- Thioglycollate broth

- Anaerobic Gas Pack system:

How the anaerobic atmosphere (or microaerophilic) is generated by gas pack system inside the anaerobic jar?

the hydrogen is generated inside the jar by placing a special Gas Pack envelope immediately before placing it in the jar, it will release hydrogen & Co_2. The presence of the Cold Catalyst in the jar allows the hydrogen released to combine with the oxygen in the jar to give strictly anaerobic condition.

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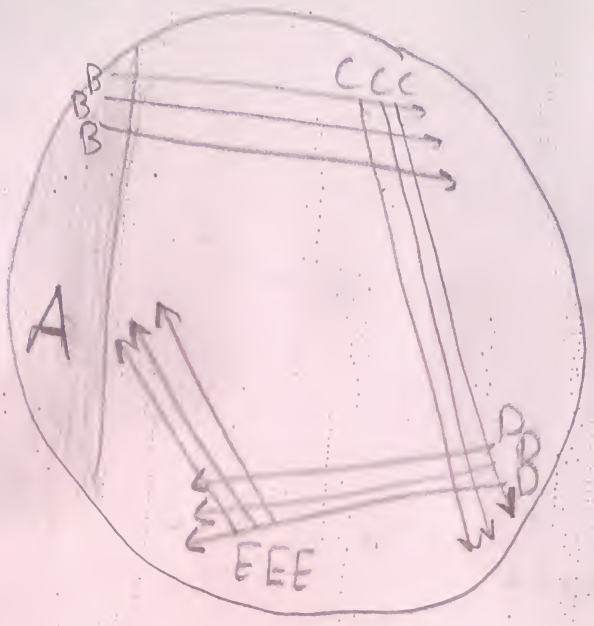
nique for isolation of bacteria
rpret antibiotic sensitivity test.

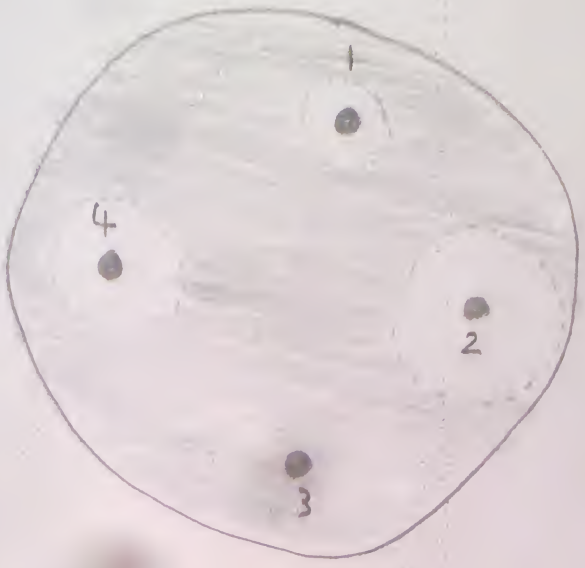
- Draw a diagrammatic description of plating out technique.



- How to get a pure culture?
  - 1ry Culture
  - 2ry Culture
  - Tertiry
  - S Shaped

  → Colony
- Pure culture is used for:
  - Identification morphology character
  - antibiotic sensitivity test
  -

gram describing it. sensitivity plate showing... and draw a
Zone of inhibition



- The plate showing a zone of inhibition around the discs No. 2 4 .....,..... and .....1.....The discs No.....,3.....show no inhibition zone.

- Complete the following table:

The antibiotic disc No.	Susceptibility of bacteria
1	Low
2	high
3	no
4	Medium

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(1): Demonstration of bacterial culture characteristics

ILOs: To examine and identify different selected culture characteristics of bacteria.

- Complete the following table (1):

Medium	Type of hemolysis	Other features	Example of grown bacteria
Inoculated blood agar plate	Plate (A) β	Catalase +ve	S. aureus
	Plate (B) α	Catalase -ve	Str. Pyogenes
	Plate (C) γ	aren't inhibited by optochin	Str. Viridans
		Catalase -ve	Str. bovis
	Plate (D)		

- Complete the following table (2):

Medium	Colour of colonies (or other features)	Explanation	Example of grown bacteria
Inoculated N. agar	Plate (A) golden yellow	endopigment	S. aureus
	Plate (B) greenish	exopigment	Pseudomonas
	Plate (C) opaque		
	Plate (D) Swarming	Motility	Proteus

- Complete the following table (3):

Medium	Colour of colonies	Explanation	Example of grown bacteria
Inoculated MacConkey	Plate (A) Pink	Lactose fermentation	E.Coli
	Plate (B) Yellow	Non-Lactose fermentation	Salmonella shigella
Inoculated MSA	Plate (C) Pink	Non-ferment of mannite	S. epidermidis
	Plate (D) Yellow	fermentation of mannite & acid Production	S. aureus
Inoculated TCBS	Plate (E) Yellow	Sucrose fermentation	1, 139 2 types of Cholera
	Plate (F) Green	Non-Sucrose fermentation	Other species of Cholera

Report(8): Demonstration of some selected biochemical reactions

ILOs: 1. To examine and identify some selected biochemical reactions.
2. To distinguish positive and negative tests.

1. **Catalase test:**
   - Principle: differentiation between Staphylococci from Streptococci

	Description of	Examples (organisms)
+ve test	H_2O_2 bubbling	Staphylococci
-ve test	no bubbling	Streptococci

2. **Oxidase test:**
   - Principle: Some bacteria eg Neisseria Produce oxidase e. which reduces the oxidase reagent (tetramethyl-P-Phenylene-diamine hydrochloride) to deep Purple Colour.

	Description of	Examples (organisms)
+ve test	deep Purple Colour	Neisseria
-ve test	Yellow	Staphylococci

3. **Indole test:**

- Principle: Demonstrate ability of bacteria to decompose A.A tryptophan in peptone to produce indole then we test for indole by Kovacs reagent

	Description of	Examples (organisms)
+ve test	Red ring	E. Coli
-ve test	Yellow ring	Klebsiella

4. **Simmonds' citrate test:**

- Principle: Demonstrate ability of certain bacteria to utilize citrate as only source of carbon

	Description of	Examples (organisms)
+ve test	blue Colour	Klebsiella
-ve test	green Colour	E. Coli

5. **Urease test:**

- Principle: Detect Production of urease enzyme in media Contain Urea & Phenol red - urease de Compose urea & release ammonia So PH alkaline → Phenol red Change to red

	Description of	Examples (organisms)
+ve test	deep pink	Proteus - Klebsiella
-ve test	Yellow	E. Coli - Salmonella

6. **Coagulase test:**

- Principle: detects production of Coagulase enzyme leads to Clotting of Plasma its Produced by S. aureus

	Description of	Examples (organisms)
+ve test	Clot	S. aureus
-ve test	not Clot	any

7. **Methyl red (MR) test:**
   - Principle: Detect Production of acid in glucose Phosphate Peptone

	Description of	Examples (organisms)
+ve test	Pink Colour	E. Coli
-ve test	Yellow Colour	Kleb Siella

8. **Voges-Proskauer (VP)test:**
   - Principle: Detect Production of acetyl methyl Carbinol & small acid in glucose phosphate Peptone during glucose fermentation

	Description of	Examples (organisms)
+ve test	Pink Colour	Kleb Siella
-ve test	Yellow Colour	E. Coli

Triple Sugar Iron agar:

- Composition:
  - Sugars (%): 0.1% glucose 1% lactose 1% sucrose ferrous sulphate - Phenol red, beef extraction
  - Indicator: Phenol red
  - Ferrous sulphate to detect: for detection H_2S
  - agar agar: - Solidification
    - Soft agar cracks on gas Production
- The **TSI** agar is poured in test tubes in the form of Slants with a deep butt
- The **medium** is of low concentration of agar (soft agar) , why?

  Cracks on gas Production

- Interpretation of TSI test:
  - Complete the following table:

Drawing of TSI pattern	Butt colour	Slant colour	H_2S and gas	Explanation	Example (organisms)
	Red	Red	no	No Carbohydrate fermenting	Pseudomonas
	Yellow	Red	no	Ferment glucose only will release small amount of acid	Shigella
	Yellow	Yellow	no	ferment lactose & or sucrose release bigamount of acid	E.Coli
	black	red	✓	fermenting glucose only with H_2S Production	Salmonella

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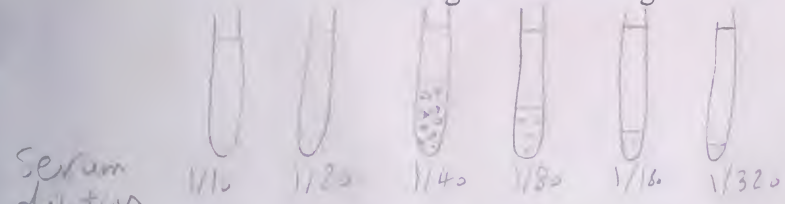
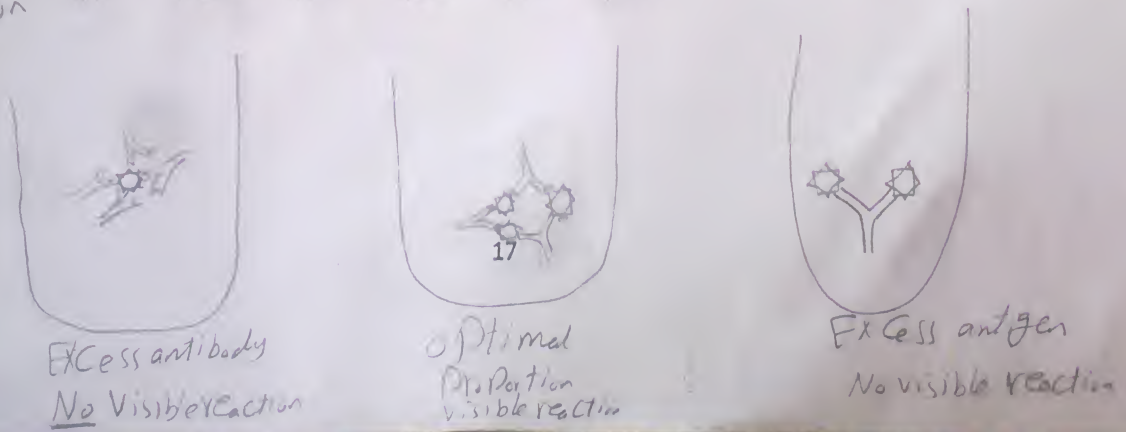
of serological diagnostic tests

ILOs: 1. To identify some selected serological tests.
2. To distinguish positive and negative tests and read the titres of positive tests.

- Tube agglutination test:
  1. Widal test:
     - Mention antigen suspensions used in this test.
       - Salmonella O antigen for 3 organism
       - H antigen of S.typhi S.ParatyphiA S.Paratyphi B
     - Examine the visible clumping at the bottom of the tubes.
     - Identify the highest dilution that shows visible agglutination (the titre):...1/320...
     - Interpretation of the test:
       +ve

  2. Brucella standard agglutination test:
     - Examine the test and determine the titre: 1/80
     - A wide range of dilution of the patients' serum are used. Why?
       to avoid Zone phenomenon
       if - antibody excess in first
       - IgA blocking antibodies
     - Draw a diagram describing the test:





3. Antistreptolysin O titre:

A test for determination of antibodies titre to streptolysin O of *Strept. pyogenes*

- The highest dilution of the patients' serum showing ..t.. the ASO titre.
- When should the test is considered positive?

titre more than 1/200

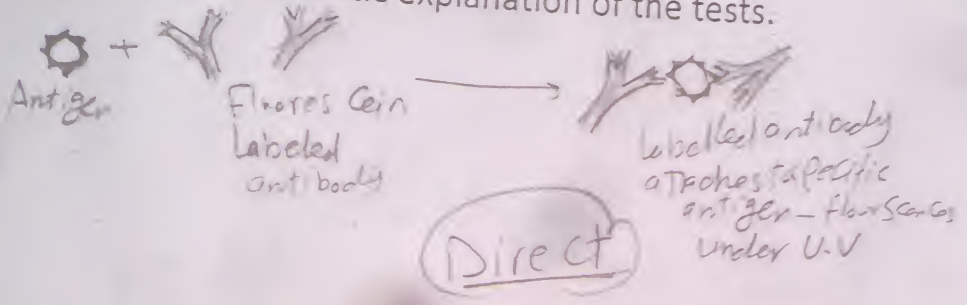
- What is the type of antigen-antibody reaction?
  - Agglutination
  - precepitation
  - Complement fixation
  - ~~immunofluorescence~~
  - ~~ELISA~~ Toxin antitoxin neutralization

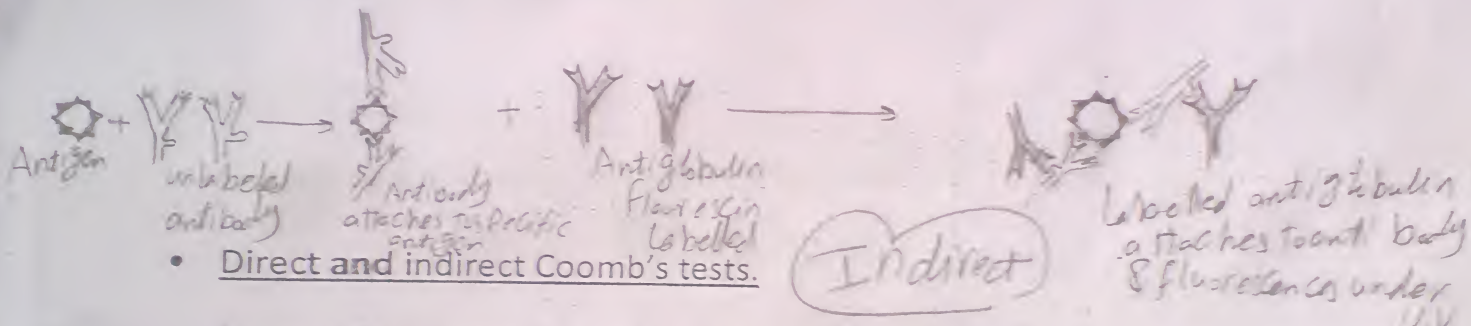
tests based on antigen – antibody reactions and some molecular techniques.

ILOs: To know and understands the principle of the tests.

- Direct and indirect florescent technique.

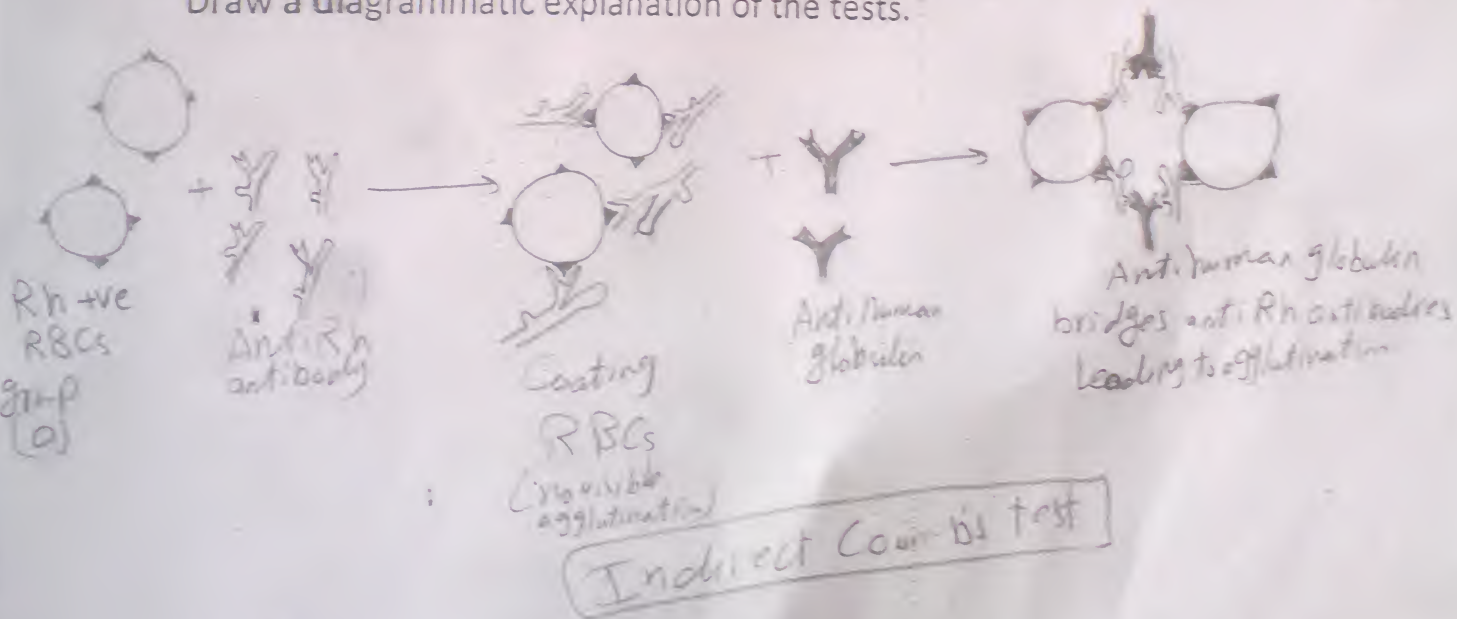
  Draw a diagrammatic explanation of the tests.





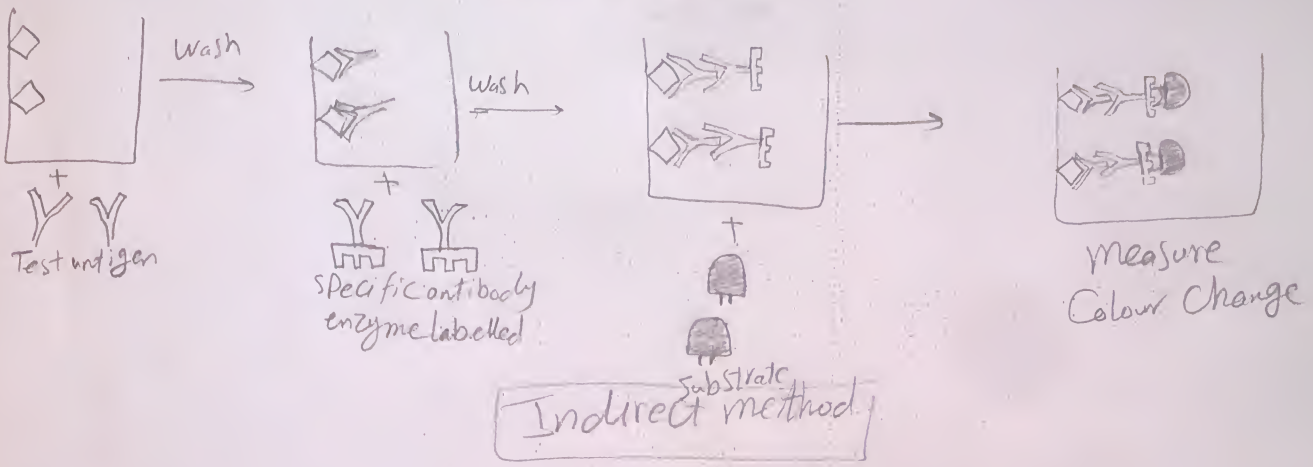
- Direct and indirect Coomb's tests.

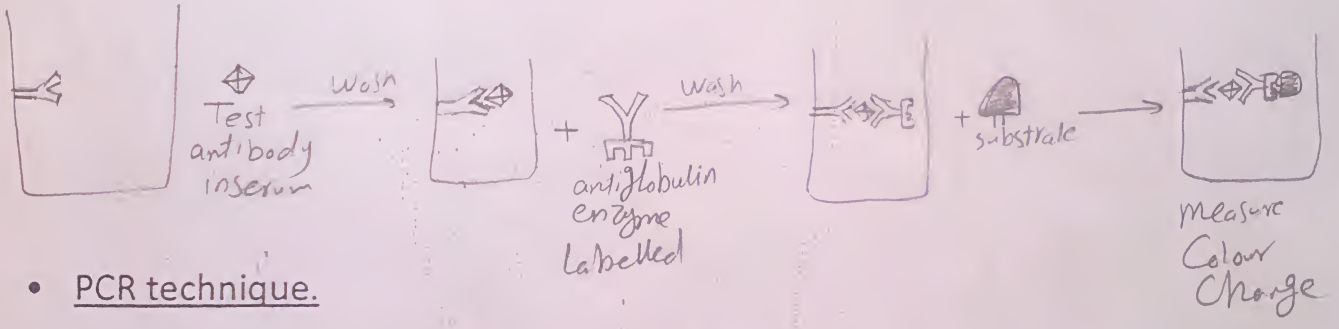
  Draw a diagrammatic explanation of the tests.



- ELISA test.

Draw a diagrammatic explanation of the test.





- PCR technique.

Draw a diagrammatic explanation of the test.